Epidemiol. Infect. (1997), **119**, 143–149. Printed in the United Kingdom © 1997 Cambridge University Press

Vibrio furnissii isolated from humans in Peru: a possible human pathogen?

A. DALSGAARD[1]*, P. GLERUP[1], L.-L. HØYBYE[1], A.-M. PAARUP[1], R. MEZA[2], M. BERNAL[2], T. SHIMADA[3] AND D. N. TAYLOR[2]

[1] *Department of Veterinary Microbiology, The Royal Veterinary and Agricultural University, DK-1870 Frederiksberg C, Denmark*
[2] *United States Naval Medical Research Inst. of Detachment, Lima, Peru*
[3] *Department of Bacteriology, National Institute of Infectious Diseases, Shinjuku-ku, Tokyo, Japan*

(*Accepted 18 March 1997*)

SUMMARY

During a cholera surveillance programme, *Vibrio furnissii* was isolated in late January and early February 1994 from stool samples collected from 14 persons of whom six had diarrhoea. The remaining eight persons were healthy family members or neighbours to cholera cases. No common source of infection was found. Strains isolated from stool samples each showed typical biochemical reactions of *V. furnissii* including gas production. Each isolate, except one, agglutinated O-antisera yielding a total of eight different serotypes. Most isolates were sensitive to 10 antibiotics tested, except to ampicillin and the vibriostatic agent O/129 (10 μg). Eight of 14 (57%) strains carried plasmids in the size range 2·6–88 kb, however, no correlation was found between antibiotic susceptibility patterns and plasmid content. Altogether, seven closely related *Hin*dIII ribotypes were observed among the 14 *V. furnissii* isolates studied. *V. furnissii* strains isolated from family members and other persons living close together often showed different ribotypes suggesting that the isolation was not associated with neighbourhood. Serotyping, plasmid profiling and ribotyping revealed a high strain diversity within *V. furnissii*, however, the importance of *V. furnissii* as an enteric pathogen remains to be elucidated.

INTRODUCTION

Vibrio spp. are natural inhabitants of aquatic environments, and most people acquire infections by exposure to such environments or to foods derived from or contaminated by them [3]. Of several established *Vibrio* spp., an increasing number are recognized to be pathogenic to human, with *Vibrio cholerae* being the most important [3].

The species name *Vibrio furnissii* was proposed in 1983 by Brenner and colleagues [1] for the biogroup 2 strains of *Vibrio fluvialis* that produced gas from the fermentation of carbohydrates [2]. The separation of *V. furnissii* from *V. fluvialis* was supported by studies of DNA relatedness [1].

V. fluvialis has been associated with sporadic cases [2, 4, 5], and it appears to have the potential to cause outbreaks of diarrhoea [3, 6]. Diarrhoea associated with *V. fluvialis* has often been connected with the consumption of seafood, especially raw shellfish [5]. *V. furnissii* has frequently been isolated from the estuarine environment but only from few human cases of diarrhoea, and the role of the organism as an enteric pathogen remains to be establish [1–3].

This paper presents the clinical status of six diarrhoea cases and eight healthy persons in Lima, Peru from whom *V. furnissii* was isolated from stool

* Author for correspondence.

samples. Bacterial specimens were characterized phenotypically and by plasmid profiling and ribotyping to elucidate whether *V. furnissii* was associated with diarrhoea and to determine any possible epidemiological relationship.

METHODS

Bacterial specimens

A cholera surveillance was carried out from February 1994 to July 1995 in Lima, Peru to evaluate the efficacy of a *V. cholerae* vaccine [7]. Stool specimens were obtained from all diarrhoeal cases detected by an active (household visits) and/or passive (hospital referrals) surveillance programme. When a cholera case was detected, stool samples were collected from family members and neighbours. All stool specimens were screened for pathogenic *Vibrio* spp. at the Microbiology Laboratory, US Naval Medical Research Institute Detachment (NAMRID), Lima, Peru using standard procedures including enrichment for 6 h in alkaline peptone water (pH 8·6) followed by plating onto thiosulfate citrate bile salt sucrose (TCBS) agar (Difco, Detroit, MI) [8].

During the surveillance period a number of non-O1 *V. cholerae* were isolated from patients with diarrhoea. We have previously reported an outbreak of diarrhoea caused by *V. cholerae* non-O1 serotypes [9]. *V. fluvialis* was frequently isolated from cases of diarrhoea throughout the surveillance period (Fig. 1). However, from 28 January to 17 February 1994, stool samples obtained from 14 persons produced growth of yellow colonies on TCBS agar which resembled *V. fluvialis* in their biochemical reactions except they were aerogenic. The isolates were characterized and identified as *V. furnissii* [3]. Haemolysis was determined on tryptic soy agar (Difco) supplemented with 5% calf blood. All media used to identify *V. furnissii* contained 1% NaCl unless specified otherwise. In addition, the API 20E assay (bioMérieux, France) was used to identify isolates suspected to be *V. furnissii* following the manufacturers recommendation for the identification of *Vibrio* species. No other pathogenic *Vibrio* spp. were recovered from stool samples of the 14 persons and none was treated with antibiotics.

V. furnissii type strain ATCC 35016 [1] and *V. fluvialis* type strain ATCC 33809 [2] previously isolated from human faeces and the marine environment, respectively, were included as reference strains in subsequent studies of *V. furnissii* isolates.

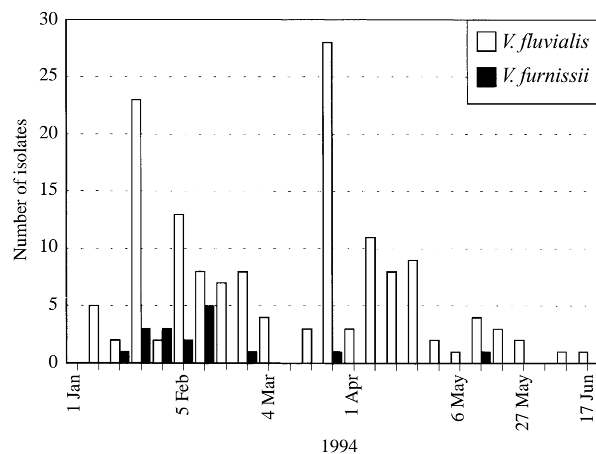

Fig. 1. Isolation of *V. furnissii* and *V. fluvialis* from patients with diarrhoea in Pampas de San Juan, Lima, Peru from January to June 1994.

Serotyping

Isolates were tested for agglutination of O serogroup antisera at the National Institute of Infectious Diseases, Tokyo, Japan, according to a combined scheme for serotyping of *V. fluvialis* and *V. furnissii* originally established by Shimada and Sakazaki in 1983 [4]. The scheme was extended by Shimada and colleagues [10] and presently covers 52 O-antigens, however, O36–O52 are unpublished (personal communication, Dr Shimada, National Institute of Infectious Diseases, Japan).

Antibiotic susceptibility testing

The 14 *V. furnissii* isolates were tested for antibiotic susceptibilities to 10 antibacterial agents by the disk diffusion method on Mueller-Hinton II Agar (Difco) containing 1% NaCl with disks (BBL, Sensi-disc, Becton, Dickinson, MD) containing (μg/disk): nalidixic acid 30, ampicillin 10, carbenicillin 100, cephalothin 30, chloramphenicol 30, doxycycline 30, gentamicin 10, norfloxacin 10, tetracycline 30 and trimethoprim/sulphamethoxazole 1·25/23·75. In addition, strains were tested for susceptibility to the vibriostatic agent (O/129) with disks containing 10 and 150 μg. Antibiotic susceptibility testing was carried out by the Bauer–Kirby method [11] and strains were recorded as either sensitive or resistant.

Isolation of plasmid DNA

Plasmid preparation was carried out using the method of Kado and Liu [12], modified by incubating the cells

at elevated pH (12·75) for 30 min at 56 °C during the lysis step [13]. Following electrophoresis, the plasmids were visualized essentially as described previously [14]. *V. cholerae* O1 strain V1075/25 containing an approximately 150 kb plasmid was used as control strain [15]. Plasmid sizes were estimated from the migration in the agarose gels relative to the migration of reference plasmids in *E. coli* strains V517 and 39R861 [16, 17] by the method of Rochelle and colleagues [18]. Repeated extraction of plasmid DNA was carried out for all isolates.

Ribotyping

Total bacterial DNA was extracted by the method of Murray and Thompson [19]. On the basis of previous studies [20, 21] and preliminary chromosomal digestion experiments using *Hin*dIII, *Eco*RI and *Bgl*I (Promega, Madison, WI), *Hin*dIII provided the best discrimination among *V. furnissii* isolates and was therefore used to digest chromosomal DNA from all strains. Ribotyping was performed by the procedure described by Dalsgaard and colleagues [13] with digoxigenin-labelled 16S and 23S rRNA probes. A 1-kb molecular weight standard (GIBCO BRL, Gaithersburg, MD) was used as a weight marker. Nylon membranes with immobilized DNA restriction fragments were hybridized, and fragments were detected colourimetrically as previously described [22].

RESULTS

The distribution of persons from whom *V. furnissii* and *V. fluvialis* were isolated from faecal specimens in Pampas de San Juan, Peru from January to June 1994 is shown in Figure 1. The clinical status of the 14 persons from whom *V. furnissii* was isolated is shown in Table 1. Five cases of diarrhoea occurred in adults and one child of either sex from at least two locations in Lima. One patient (patient no. 3) suffered profuse diarrhoea with traces of blood. However, eight persons did not show signs of diarrhoea. Unfortunately, stool samples were analysed for pathogenic *Vibrio* spp. and enterotoxigenic *E. coli* only, hence, we do not know whether a second pathogen could have been the primary cause of the diarrhoea.

All 14 *V. furnissii* strains produced yellow colonies on TCBS agar, were motile at 37 °C, and showed identical characteristics including the production of arginine dihydrolase, gas and indole. The strains showed growth in broths containing 3, 6 and 8 % NaCl, but not in broths containing 0 and 10 % NaCl. All strains were sensitive to O/129 (150 μg) but showed resistance to O/129 (10 μg). The reference strains of *V. furnissii* and *V. fluvialis* showed similar phenotypic characteristics compared with the clinical *V. furnissii* isolates, except that *V. fluvialis* showed a positive cellobiose test and did not produce gas. All isolates produced opaque colonies on tryptic soy agar (Difco).

In the API 20E system three 7-digit profiles, 3246126, 3046126 and 3006126, were shown for the 14 *V. furnissii* strains isolated in Peru (Table 2). Variation was shown in the citrate test and the production of indole with three and nine strains showing positive reactions, respectively. All isolates showed a positive ONPG test, hydrolysed gelatin, produced acid from mannitol and arabinose but not from rhamnose. The reference strain of *V. fluvialis* ATCC 33809 showed profile 3004127. However, *V. furnissii* and *V. fluvialis* are not among the *Vibrio* species included in the matrix of the API 20E version 3.1 software recommended by the manufacturer for bacterial identification (bioMérieux), thus identification scores were not determined. In contrary to the API 20E assay where three strains showed a positive reaction in the citrate test, all strains showed a positive reaction when tested for citrate utilization by traditional biochemical testing. False negative results of the citrate test in the API 20E assay have been reported previously for *Vibrio vulnificus* [23]. In addition, gas production may not be detected in the API 20E assay. The results of our study suggest that the reactions in the API 20E assay are inadequate for the identification of *V. furnissii*.

The results of the serogrouping are shown in Table 2. Each of the *V. furnissii* isolates, except strain VIG 2171, agglutinated with antiserum demonstrating a total of eight different serotypes [10]. Serotype O11 was predominant and shown by four isolates.

Twelve (86 %) of the clinical isolates showed resistance to ampicillin and three isolates exhibited resistance to cephalothin (Table 2). Strain VIG 1143 was the only strain showing resistance to trimethoprim/sulphamethoxazole whereas strain VIG 2171 was the only strain exhibiting resistance to nalidixic acid. No strains were multiply resistant ($\geqslant$ 3 antibiotics). All isolates showed resistance to low dose vibriostaticum (10 μg) but all were sensitive to high dose vibriostaticum (150 μg).

Table 1. *Clinical status of persons from whom* Vibrio furnissii *was isolated from stool samples*

Strain no./ patient no.	Age/ sex*	Date of isolation	Type of illness	Days of diarrhoea
CIT 69/1	18/M	3 Feb 1994	Diarrhoea	Unknown
CIT 143/2	19/M	3 Feb 1994	Diarrhoea	Unknown
VIG 1052/3	20/M	28 Jan 1994	Diarrhoea with blood	3
VIG 1143/4	36/F	31 Jan 1994	Diarrhoea with dehydration	5
VIG 1174/5	34/F	31 Jan 1994	Diarrhoea	1
VIG 1184/6	35/M	31 Jan 1994	Well	0
VIG 1301/7	2/F	2 Feb 1994	Well	0
VIG 1844/8	13/F	11 Feb 1994	Well	0
VIG 1869/9	4/M	11 Feb 1994	Well	0
VIG 2042/10	40/F	14 Feb 1994	Well	0
VIG 2048/11	35/M	14 Feb 1994	Well	0
VIG 2171/12	5/M	16 Feb 1994	Diarrhoea	7
VIG 2318/13	34/M	17 Feb 1994	Well	0
VIG 2265/14	29/F	17 Feb 1994	Well	0

* Age is indicated as number of years. Sex: M, male; F, female.

Table 2. *Phenotypic and genotypic characterization of* 14 Vibrio furnissii *strains isolated in Lima, Peru in 1994*

Strain	Date of isolation (1994)	O serogroup*	API20E profile	Antibiogram†	Plasmid size (kb)	Ribotype‡
CIT 69	3 Feb	O50	3246126	Am; Carb; Ceph; O/129	70	VFU1
CIT 143	3 Feb	O50	3246126	Am; Carb; Ceph; O/129	70	VFU1
VIG 1052	28 Jan	O11	3046126	O/129	—§	VFU6
VIG 1143	31 Jan	O12	3006126	Am; TR; O/129	62	VFU2
VIG 1174	31 Jan	O21	3046126	Am; Ceph; O/129	—	VFU5
VIG 1184	31 Jan	O10	3006126	Am; O/129	—	VFU2
VIG 1301	2 Feb	O46	3246126	Am; O/129	88	VFU2
VIG 1844	11 Feb	O46	3046126	O/129	—	VFU4
VIG 1869	11 Feb	O21	3006126	Am; O/129	88; 70; 26; 4·6	VFU7
VIG 2042	14 Feb	O11	3006126	Am; O/129	2·6	VFU3
VIG 2048	14 Feb	O11	3006126	Am; O/129	2·6	VFU3
VIG 2171	16 Feb	Unknown	3046126	Am; Nal; O/129	—	VFU2
VIG 2318	17 Feb	O5	3046126	Am; O/129	—	VFU4
VIG 2265	17 Feb	O11	3046126	Am; O/129	3·4	VFU2
ATCC 35016‖ (*V. furnissii*)		O6	3006126	O/129	88	VFU8
ATCC 33809** (*V. fluvialis*)		O7	3004127	O/129	—	VFL1

* O serogroup designation according to the scheme original published by Shimada and Sakazaki in 1983 [4].
† Am, ampicillin; Carb, carbenicillin; Nal, nalidixic acid; Ceph, cephalothin; TR, trimethoprim/sulphamethoxazole; O/129, vibriostatic agent low (10 μg).
‡ Ribotypes were established using the enzyme *Hin*dIII, VFU: *V. furnissii*; VFL: *V. fluvialis*.
§ No plasmids were found.
ATCC, American Type Culture Collection [1]; ** NCIMB, National Culture of Industrial and Marine Bacteria, Aberdeen, Scotland.

Analysis of the plasmid content of the *V. furnissii* strains revealed that 8/14 (57%) strains carried plasmids including strain VIG 1869 which carried four plasmids (Fig. 2, Table 2). Strains CIT 69, CIT 143 and VIG 1869 carried an approximately 70 kb plasmid whereas strains VIG 1301, VIG 1869 and

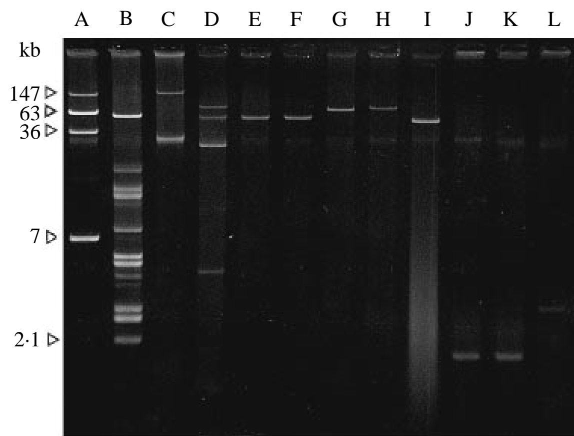

Fig. 2. Plasmid profiles of *Vibrio furnissii*. Lanes: A, *Escherichia coli* 39R 861 (four plasmids ranging from 147 kb to 6·9 kb); B, *E. coli* V517 (eight plasmids ranging from 54 kb to 2·0 kb); C, *Vibrio cholerae* O1 strain V1075/25; D, VIG 1869; E, CIT 143; F, CIT 69; G, VIG 1301; H, ATCC 35016; I, VIG 1143; J, VIG 2042; K, VIG 2048; L, VIG 2265.

ATCC 35016 harboured a 88 kb size plasmid. The control strain V1075/25 contained an approximately 150 kb plasmid. There did not appear to exist any correlation between antibiotic susceptibility patterns and plasmid content.

Altogether, seven *Hin*dIII ribotypes were observed among the 14 *V. furnissii* clinical isolates studied (Fig. 3). Patterns were considered to be different when there was a difference of one band between isolates. The ribotypes appeared closely related as all isolates presented eight common fragments within the size ranges of 0·5 kb; 1·7–2·9 kb and 4 kb. Fragments within the size range 4·5–7·0 kb showed the highest degree of variability. Ribotypes were designated VFU (*V. furnissii*) followed by an arbitrary number (Table 2). Five isolates showed an identical ribotype VFU2 whereas each of the ribotypes VFU1 and VFU3 were demonstrated by two isolates. Isolates showing VFU2 agglutinated three different antisera (Table 2). Only limited correlation was demonstrated between O-serogroup designations and ribotypes.

The *V. furnissii* type strain ATCC 35016 showed an unique ribotype VFU8 closely related to the ribotypes demonstrated by the other *V. furnissii* isolates. A unique ribotype VFL1 shown by *V. fluvialis* type strain ATCC 33809 presented several identical

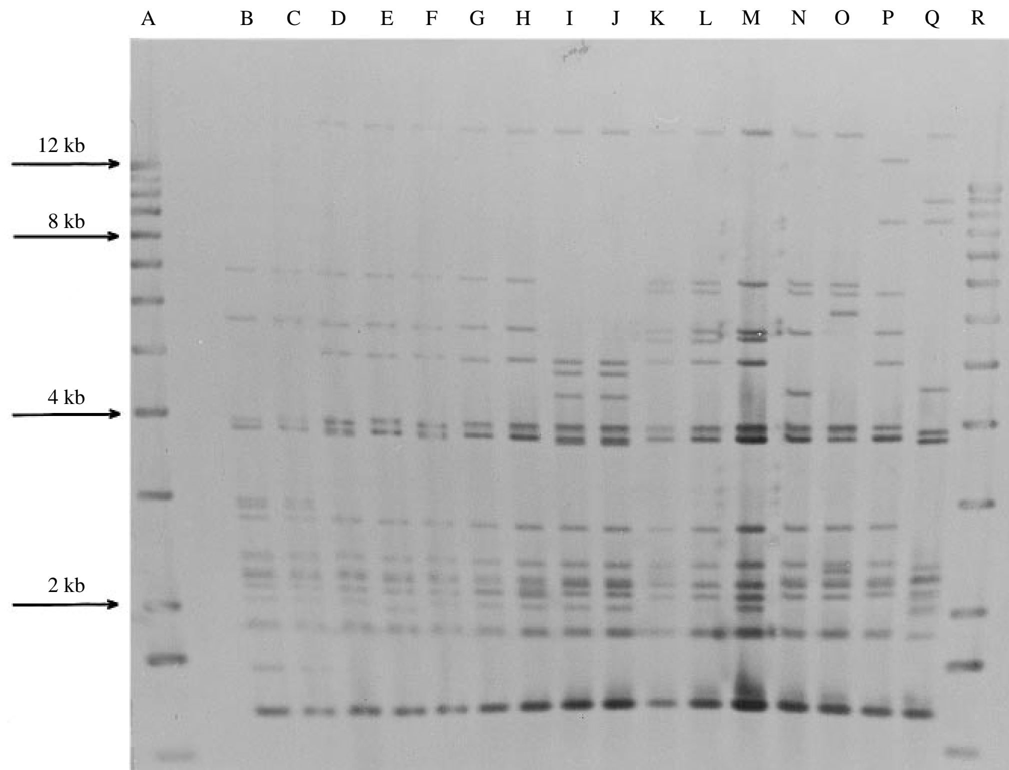

Fig. 3. *Hin*dIII ribotypes of *Vibrio furnissii* isolates recovered from 14 patients with diarrhoea in Lima, Peru. Explanation of lanes include strain designation and ribotype. Lanes: A, 1 kb molecular weight standard; B, CIT 69, type VFU1; C, CIT 143, type VFU1; D, VIG 1143, type VFU2; E, VIG 1184, type VFU2; F, VIG 1301, type VFU2; G, VIG 2171, type VFU2; H, VIG 2265, type VFU2; I, VIG 2042, type VFU3; J, VIG 2048, type VFU3; K, VIG 1844, type VFU4; L, VIG 2318, type VFU4; M, VIG 1174, type VFU5; N, VIG 1052, type VFU6; O, VIG 1869, type VFU7; P, ATCC 35016 (*V. furnissii*), type VFU8; Q, ATCC 33809 (*V. fluvialis*), type VFL1; R, 1 kb molecular weight standard.

fragments compared with the *V. furnissii* ribotypes (Fig. 3).

DISCUSSION

In this study we present data on the isolation of *V. furnissii* from stool samples collected from 14 persons of whom only six had diarrhoea. The remaining eight persons were healthy family members or neighbours to cholera cases. The *V. furnissii* strains were isolated primarily in January and February 1994 with a few sporadic isolations during the rest of the year. January and February are the warm summer months in Peru and a time when cholera as well as other bacterial causes of diarrhoea are on the increase. The characterization of the *V. furnissii* strains showed a high degree of diversity and did not reveal any epidemiological relationship between the persons from whom *V. furnissii* was isolated. If *V. furnissii* was the cause of the diarrhoea the low case to infection ratio suggests a moderate virulence, however, the exact role of *V. furnissii* as an enteric pathogen remains to be elucidated. Since previous studies have shown production of enterotoxin or enterotoxin-like molecules by *V. fluvialis* similar studies could be performed with *V. furnissii* [24, 25].

From the patient data we were not able to determine the transmission of the *V. furnissii* and no evidence of a common source of infection was found. However, since *V. furnissii* is often found in water and seafood, such samples may have been vehicles of transmission [26].

All isolates were found susceptible to the majority of antibiotics tested. It was not surprising that most isolates showed resistance to ampicillin as the production of β-lactamases is common among *Vibrio* species [3]. In the present study, all isolates showed resistance to low dose vibriostaticum (10 μg) but all were sensitive to high dose vibriostaticum (150 μg). A high prevalence of resistance to the vibriostatic agent O/129 (150 μg) has been reported previously for *V. furnissii* [2, 3].

Although several isolates carried relatively large plasmids they did not seem to encode antibiotic resistance, neither did there appear to be any association between plasmid content and association with diarrhoea. Plasmid content in *V. furnissii* has not been reported previously and their importance remains to be determined [1].

There appeared to be only limited correlation between certain ribotypes and their association with diarrhoea as five strains showing ribotype VFU2 were recovered from patients with diarrhoea and healthy controls. *V. furnissii* strains isolated from family members and other persons living close together often showed different ribotypes suggesting that the isolation was not associated with neighbourhood. However, two *V. furnissii* strains isolated from patients 1 and 2 both with diarrhoea showed an identical ribotype VFU1. In addition, the two strains showed identical O serogroup, antibiogram and plasmid profile.

The association of *V. furnissii* with diarrhoea remains to be established, thus, we are continuing our studies to determine the role of *V. furnissii* as an enteric pathogen.

ACKNOWLEDGEMENTS

We are grateful to Ines Pineda, Betzabe Alarcon and Hector Verastegui for their help in establishing the surveillance system and to the study nurses and hospital staff for their support. We wish to thank Nilda Gadea for her work in the laboratory. At the Royal Veterinary and Agricultural University, Denmark we are grateful for the technical assistance of Anita Forslund and for critical review of the manuscript by David J. Platt. Financial support for the study were provided by the Panamerican Health Organization and by the Diarrhoeal Disease Control Programme of the World Health Organization. Anders Dalsgaard was supported by a grant from the Danish Council for Development Research, Danish International Development Assistance (DANIDA), fellowship no. 90810.

REFERENCES

1. Brenner DJ, Hickman-Brenner FW, Lee JV, et al. *Vibrio furnissii* (formerly aerogenic biogroup of *Vibrio fluvialis*), a new species isolated from human feces and the environment. J Clin Microbiol 1983; **18**: 816–24.
2. Lee JV, Shread P, Furniss AL. Taxonomy and description of *Vibrio fluvialis* sp. nov. (synonym group F vibrios, group EF6). J Appl Bacteriol 1981; **50**: 73–94.
3. Kelly MT, Hickman-Brenner FW, Farmer III, JJ. Vibrio. In: Balows A, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy HJ, eds. Manual of clinical microbiology. Washington, DC: ASM Press, 1991: 384–95.
4. Klontz KC, Desenclos J-JA. Clinical and epidemiological features of sporadic infections with *Vibrio*

fluvialis in Florida, USA. J Diarrhoeal Dis Res 1990; **8**: 24–6.
5. Levine WC, Griffin PM. *Vibrio* infections on the Gulf Coast: results of first year of regional surveillance. J Infect Dis 1993; **167**: 479–83.
6. Huq MI, Alam AKMJ, Brenner DJ, Morris GK. Isolation of *Vibrio*-like group, EF-6 from patients with diarrhea. J Clin Microbiol 1980; **11**: 621–4.
7. Sanchez JL, Vasquez B, Begue RE, et al. Protective efficacy of oral, whole cell/recombinant B subunit cholera vaccine in Peruvian military recruits. Lancet 1994; **344**: 1273–6.
8. Sakazaki R. Bacteriology of vibrio and related organisms. In: Barua D, Greenough WR, eds. Cholera. New York: Plenum Publishing Company, 1992: 37–55.
9. Dalsgaard A, Albert MJ, Taylor DN, et al. Characterization of *Vibrio cholerae* non-O1 serogroups obtained from an outbreak of diarrhea in Lima, Peru. J Clin Microbiol 1995; **33**: 2715–22.
10. Shimada T, Kosako Y, Inoue K, et al. *Vibrio fluvialis* and *V. furnissii* serotyping scheme for international use. Curr Microbiol 1994; **22**: 335–7.
11. Bauer AW, Kirby MM, Sherris JC, Turch M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Path 1966; **36**: 493–6.
12. Kado CI, Liu ST. Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol 1981; **145**: 1365–73.
13. Dalsgaard A, Mortensen HF, Mølbak K, Dias F, Serichantalergs O, Echeverria P. Molecular characterization of *Vibrio cholerae* O1 strains isolated during cholera outbreaks in Guinea-Bissau. J Clin Microbiol 1996; **34**: 1189–92.
14. Olsen JE, Larsen JL. Restriction fragment length polymorphism of the *Vibrio anguillarum* serovar O1 virulence plasmid. Appl Environ Microbiol 1990; **56**: 3130–2.
15. Tabtieng R, Wattanasri S, Echeverria P, et al. An epidemic of *V. cholerae* El Tor Inaba resistant to several antibiotics with a conjugative group C plasmid coding for type II dihydrofolate reductase in Thailand. Am J Trop Med Hyg 1989; **41**: 680–6.
16. Macrina FL, Kopecko DJ, Jones KR, Ayers DF, McCowen SK. A multiple plasmid-containing *Escherichia coli* strain: convenient source of size reference plasmid molecules. Plasmid 1987; **1**: 417–20.
17. Threlfall DJ, Rowe B, Ferguson JL, Ward LR. Characterization of plasmids conferring resistance to gentamicin and apramycin in strains of *Salmonella typhimurium* phage type 204c isolated in Britain. J Hyg 1986; **97**: 419–26.
18. Rochelle PA, Fry JC, Day MJ, Bale MJ. An accurate method for estimating sizes of small and large plasmids and DNA fragments by gel electrophoresis. J Gen Appl Microbiol 1985; **132**: 53–9.
19. Murray MG, Thompson WF. Rapid isolation of high molecular weight DNA. Nucl Acids Res 1980; **8**: 4321–5.
20. Dalsgaard A, Echeverria P, Larsen JL, Siebeling R, Serichantalergs O, Huss HH. Application of ribotyping for differentiating *Vibrio cholerae* non-O1 isolates from shrimp farms in Thailand. Appl Environ Microbiol 1995; **61**: 245–51.
21. Dalsgaard A, Frimodt-Møtleig N, Bruun B, Høi L, Larsen JL. Clinical manifestations and epidemiology of *Vibrio vulnificus* infections in Denmark. Euro J Clin Microbiol Infect Dis 1996; **15**: 227–32.
22. Popovic T, Bopp CA, Olsvik Ø, Wachsmuth K. Epidemiologic application of a standardized ribotype scheme for *Vibrio cholerae* O1. J Clin Microbiol 1993; **31**: 2474–82.
23. Dalsgaard A, Dalsgaard I, Høi L, Larsen JL. Comparison of the API20E assay and an alkaline phosphatase-labelled DNA probe for identification of *Vibrio vulnificus* isolated from Danish coastal waters. Lett Appl Microbiol 1996; **22**: 184–8.
24. Lockwood DE, Kreger AS, Richardson SH. Detection of toxins produced by *Vibrio fluvialis*. Infect Immun 1982; **35**: 702–8.
25. Nishibuchi M, Seidler RJ. Medium-dependent production of extracellular enterotoxins by non-O1 *Vibrio cholerae*, *Vibrio mimicus*, and *Vibrio fluvialis*. Appl Environ Microbiol 1983; **45**: 228–31.
26. Lee JV, Donovan TJ, Furniss AL. Characterization, taxonomy, and emended description of *Vibrio metschnikovii*. Int J Sys Bacteriol 1978; **28**: 99–111.